Targeting multidrug resistance in cancer

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Abstract | Effective treatment of metastatic cancers usually requires the use of toxic chemotherapy. In most cases, multiple drugs are used, as resistance to single agents occurs almost universally. For this reason, elucidation of mechanisms that confer simultaneous resistance to different drugs with different targets and chemical structures — multidrug resistance — has been a major goal of cancer biologists during the past 35 years. Here, we review the most common of these mechanisms, one that relies on drug efflux from cancer cells mediated by ATP-binding cassette (ABC) transporters. We describe various approaches to combating multidrug-resistant cancer, including the development of drugs that engage, evade or exploit efflux by ABC transporters.

Anticancer drugs can fail to kill cancer cells for various reasons. Drugs are usually given systemically and are therefore subject to variations in absorption, metabolism and delivery to target tissues that can be specific to individual patients. Tumours can be located in parts of the body into which drugs do not easily penetrate, or could be protected by local environments due to increased tissue hydrostatic pressure or altered tumour vasculature.

By analogy to the study of antibiotic resistance in microorganisms, research on drug resistance in cancer has focused on cellular resistance due to either the specific nature and genetic background of the cancer cell itself, or the genetic changes that follow toxic chemotherapy. Until recently, the primary method for identifying mechanisms of multidrug resistance (MDR) was to select surviving cancer cells in the presence of cytotoxic drugs and use cellular and molecular biology techniques to identify altered genes that confer drug resistance on naive cells. Such studies indicate that there are three major mechanisms of drug resistance in cells: first, decreased uptake of water-soluble drugs such as folate antagonists, nucleoside analogues and cisplatin, which require transporters to enter cells; second, various changes in cells that affect the capacity of cytotoxic drugs to kill cells, including alterations in cell cycle, increased repair of DNA damage, reduced apoptosis and altered metabolism of drugs; and third, increased energy-dependent efflux of hydrophobic drugs that can easily enter the cells by diffusion through the plasma membrane.

Of these mechanisms, the one that is most commonly encountered in the laboratory is the increased efflux of a broad class of hydrophobic cytotoxic drugs that is mediated by one of a family of energy-dependent transporters, known as ATP-binding cassette (ABC) transporters. First described in the 1970s (BOX 1), several members of the ABC transporter family, such as P-glycoprotein (Pgp, also known as ABCB1 or MDR1), can induce MDR. The broad substrate specificity and the abundance of ABC transporter proteins might explain the difficulties faced during the past 20 years in attempting to circumvent ABC-mediated MDR *in vivo*. Cancer pharmacologists have worked to develop drugs that either evade efflux or inhibit the function of efflux transporters, and although progress in this area has been slow, the rationale for this approach is still strong and suggestions for future directions in this field are included in this review.

Recently, bioinformatic approaches, taking advantage of large drug databases tested across well-characterized cell lines, have allowed the identification of several potential cytotoxic substrates recognized by different ABC transporters. In addition, pharmacokinetic analyses and the study of knockout mice have revealed important roles of several ABC transporters in the absorption, excretion and distribution of drugs. ABC transporters are essential for many cellular processes that require the transport of substrates across cell membranes. Therefore, ABC transporters have an important role in drug discovery and development in several areas, including multidrug-resistant cancer and drug targeting to specific compartments.

The ABC transporter family

ABC transporters, named after their distinctive ATP-binding cassette domains, are conserved proteins that typically translocate solutes across cellular membranes[1]. The functional unit of an ABC transporter contains two transmembrane domains (TMDs) and two nucleotide

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doi:10.1038/nrd1984

Box 1 | Discovery of ABC transporters involved in multidrug resistance

In 1973, Dano[13] noted the active outward transport of daunomycin in multidrug-resistant Ehrlich ascites tumour cells. Subsequent work showed that the 'reduced drug permeation' in multidrug-resistant cells is associated with the presence of a cell-surface glycoprotein, termed P-glycoprotein (Pgp)[127]. Based on the presence of specific conserved sequences, Pgp was recognized to be an ATP-binding cassette (ABC) transporter protein and was proposed to function as an efflux pump[128,129–132]. A decade later, a human small-cell lung cancer cell line (H69), showing resistance to doxorubicin without increasing expression of Pgp, was identified[133]. Similar to cells overexpressing Pgp, H69-derivatives showed a combined drug accumulation defect and cross-resistance to a broad range of anticancer agents, including anthracyclines, vinca alkaloids and epipodophyllotoxins[134,135]. Analysis indicated the increased expression of a novel ABC transporter, termed MRP1 (multidrug resistance-associated protein 1)[136]. This finding also suggested that a more systematic approach could be used to discover additional Pgp-independent mechanisms of drug resistance. Using the Pgp-inhibitor verapamil in conjunction with cytotoxic agent selection resulted in the discovery of a third ABC transporter, named ABCG2 (also known as mitoxantrone resistance protein (MXR) and breast cancer resistance protein (BCRP))[137–139].

(ATP)-binding domains (NBDs). Transporters such as ABCG2 (also known as mitoxantrone-resistance protein (MXR) or breast cancer resistance protein (BCRP)) that contain only a 'half set' (one TMD and one NBD) form dimers to generate a 'full' transporter[2]. Structures of bacterial ABC transporter proteins suggest that the two NBDs form a common binding site where the energy of ATP is harvested to promote efflux through a pore that is delineated by the transmembrane helices[3].

The human genome contains 48 genes that encode ABC transporters, which have been divided into seven subfamilies labelled A–G[4]. Diverse substrates are translocated by ABC transporters, ranging from chemotherapeutic drugs to naturally occurring biological compounds. Although several members of the superfamily have dedicated functions involving the transport of specific substrates, it is becoming increasingly evident that the complex physiological network of ABC transporters has a pivotal role in host detoxification and protection of the body against xenobiotics. This role is revealed by the tissue distribution of ABC transporters, which are highly expressed in important pharmacological barriers, such as the brush border membrane of intestinal cells, the biliary canalicular membrane of hepatocytes, the lumenal membrane in proximal tubules of the kidney and the epithelium that contributes to the blood–brain barrier (BBB) (FIG. 1).

Traditionally, the absorption, distribution, metabolism, excretion and/or toxicity (ADMET) of a drug were thought to be governed by the physicochemical properties of the molecule, protein binding and/or biotransformation[5]. The capacity of transport proteins to reduce oral bioavailability and alter tissue distribution has obvious implications for pharmaceutical drug design. Indeed, the identification of transporters that influence the disposition and safety of drugs has become a new challenge for drug discovery programmes. It is essential to know, first, whether drugs can freely cross pharmacological barriers or whether their passage is restricted by ABC transporters; and, second, whether drugs can influence the passage of other compounds through the inhibition of ABC transporters. Consequently, the evaluation of transport susceptibility of drug candidates has become an important step in the development of novel therapeutics, and the pharmaceutical industry has adopted routine evaluation of Pgp susceptibility in the drug discovery process (BOX 2).

Generation of mice deficient in the *mdr1a* (*abcb1a*) and *mdr1b* (*abcb1b*) genes, or both, has provided a valuable tool for the assessment of the contribution of Pgp to drug disposition *in vivo*[6]. Surprisingly, *mdr1a/1b* double knockout mice are viable and fertile — almost indistinguishable from their wild-type littermates, suggesting that pharmacological modulation of human Pgp could represent a safe and effective strategy to thwart multidrug-resistant cancers. The AUC (area under the plasma concentration versus time curve) of orally administered taxol was found to be significantly higher in the double knockout mice, indicating that Pgp expression at the intestinal lumen can limit oral drug bioavailability[7]. Further analysis of the knockout animals has demonstrated that the absence of Pgp has a profound effect on the tissue distribution of substrate compounds. So, if a drug is subject to Pgp-mediated efflux, its pharmacokinetic profile will be substantially altered by the use of Pgp inhibitors. Consistent with its high expression in brain capillary cells, Pgp also presents a barrier to hydrophobic compounds that would otherwise penetrate the BBB by passive diffusion. Pgp can thereby reduce the efficacy of agents targeted to the central nervous system (CNS) to treat epilepsy, central infections (such as HIV) or brain tumours[8]. Penetration of CNS-targeted compounds through the BBB can be estimated by comparing the brain-to-plasma ratios of drugs in Pgp-deficient mice to those of normal mice (FIG. 2). However, *in vivo* studies are not compatible with high-throughput screening (HTS) of drugs, and the knockout mouse system can provide misleading information, because there are significant species differences between the substrate specificities of human and mouse Pgp[9].

ABC transporters and *in vitro* MDR

Fulfilling their role in detoxification, several ABC transporters have been found to be overexpressed in cancer cell lines cultured under selective pressure (BOX 1). So far, tissue culture studies have consistently shown that the major mechanism of MDR in most cultured cancer cells involves Pgp, multidrug resistance associated-protein 1 (MRP1, also known as ABCC1) or ABCG2. However, cells selected to be resistant to various cytotoxic agents were found to overexpress additional ABC transporters, and several more were found to confer drug resistance in transfection studies. Current understanding indicates that at least 12 ABC transporters from four ABC subfamilies have a role in the drug resistance of cells maintained in tissue culture (FIG. 3).

ABCB subfamily. Pgp, a member of the ABCB subfamily, stands out among ABC transporters by conferring the strongest resistance to the widest variety of compounds. Pgp transports drugs that are central to most chemotherapeutic regimens, including (but certainly not limited to) vinca alkaloids, anthracyclines, epipodophyllotoxins and taxanes (for a comprehensive review see REF. 10). Pgp is normally expressed in the transport epithelium of the

AUC
The AUC is a measure of drug exposure, derived from the plasma drug concentration depicted as a function of time. It is used to determine pharmacokinetic parameters, such as clearance or bioavailability, and provides guidelines for dosing and comparing the relative efficiency of different drugs.

liver, kidney and gastrointestinal tract, at pharmacological barrier sites, in adult stem cells and in assorted cells of the immune system[11,12].

In the first study that described MDR, it was also shown that sensitization of resistant cells was achievable with modulators that prevent the export of cytotoxic drugs[13]. A later finding revealed that *in vitro* and *in vivo* resistance of P388/VCR cells to vincristine was reversible with verapamil, which immediately suggested the possible therapeutic use of inhibitors to improve the efficacy of chemotherapy substrates of Pgp[14]. Pgp-mediated drug transport is modulated by a wide range of agents. Indeed,

Figure 1 | **Summary of the pharmacological role of ATP-binding cassette transporters.** ATP-binding cassette (ABC) transporters act to prevent the absorption of orally ingested or airborne toxins, xenobiotics or drugs. Highly sensitive compartments, such as the brain, foetus or testes are protected by additional barriers. Enterohepatic circulation, as well as the excretion of compounds, is regulated by ABC transporters in the liver, gastrointestinal (GI) tract and the kidney. Although the systemic localization of ABC transporters at absorptive barriers provides an effective means to protect against dietary toxins, it also decreases the bioavailability of orally administered drugs and reduces drug disposition to physiological sanctuaries[152]. BBB, blood–brain barrier; BCSFB, blood–cerebrospinal fluid barrier; CSF, cerebrospinal fluid.

Box 2 | **Assessment of susceptibility to transport by P-glycoprotein**

It has been a challenge to find reliable cell-based or biochemical tools that enable rapid analysis of susceptibility of drug candidates to transport by P-glycoprotein (Pgp) in the pharmaceutical setting. Pgp-mediated transport is coupled to ATP hydrolysis, which is often stimulated by transported substrates[10,140]. To determine whether a candidate drug is a substrate or inhibitor of Pgp, measurement of ATPase activity can be carried out in a high-throughput manner using isolated membrane vesicles from cells expressing high concentrations of Pgp[141]. However, there are substrates and inhibitors that have little effect on the Pgp-mediated ATPase activity. Consequently, the susceptibility of compounds to Pgp-mediated transport is usually evaluated directly in intact cell systems, using cells that overexpress Pgp. *In vivo*, drugs have to cross pharmacological barriers to be absorbed, distributed or excreted. This transcellular movement is best modelled by monolayer efflux assays. In these assays, polarized epithelial or endothelial cells expressing various ATP-binding cassette transporters are grown on semipermeable filters. Pgp, localized on the apical surface of the cells, reduces transport in the apical-to-basolateral direction (that is, absorption from the gastrointestinal lumen to the blood) and increases transport of drug substrates in the basolateral to apical direction (FIG. 2). This system provides evaluation of direct transport and is widely used for the assessment of Pgp susceptibility.

due to the promiscuity of the transporter, it has been relatively easy to find non-toxic, high-affinity substrates that block transport in a competitive or non-competitive manner[15]. Inhibitors of Pgp and other transporters are extensively discussed later in this article.

The two additional members of the ABCB subfamily implicated in drug resistance are normally expressed in the liver: ABCB11 ('sister of Pgp'[16,17]), a bile salt transporter, and ABCB4 (MDR3), a phosphatidylcholine flippase[18,19]. Mutations in the genes encoding these proteins cause various forms of progressive familial intrahepatic cholestasis[20]. Transfection of ABCB11 into cells mediates paclitaxel resistance[21], and MDR3 has been shown to promote the transcellular transport of several Pgp substrates, such as digoxin, paclitaxel and vinblastine[22].

ABCC subfamily. Whereas Pgp transports unmodified neutral or positively charged hydrophobic compounds, the ABCC subfamily members (the MRPs) also transport organic anions and Phase II metabolic products. Indeed, this synergism between the efflux systems and the metabolizing/conjugating enzymes provides a formidable alliance for drug elimination. In addition to the MDR-like core structure consisting of two NBDs and two TMDs, MRPs are composed of additional domains. ABCC1, ABCC2, ABCC3, ABCC6 and ABCC10 contain an amino (N)-terminal membrane-bound region connected to the core by a cytoplasmic linker. The four remaining members (ABCC4, ABCC5, ABCC11 and ABCC12) lack the N-terminal TMD (but not the linker region, which is characteristic of the subfamily[23]).

ABCC1 (widely known as MRP1) is expressed in a wide range of tissues, clinical tumours[24] and cancer cell lines[25]. MRP1 confers resistance to several hydrophobic compounds that are also Pgp substrates (FIG. 3). In addition, like other members of the ABCC subfamily, MRP1 can export glutathione (GSH), glucuronate or sulphate conjugates of organic anions. MRP1 homologues implicated in resistance to anticancer agents include ABCC2 (MRP2), ABCC3 (MRP3), ABCC6 (MRP6) and ABCC10 (MRP7).

In contrast to most ABCC subfamily members, which are typically expressed in basolateral membranes, MRP2 is localized in the apical membranes of polarized cells, such as hepatocytes and enterocytes. So, MRP2 has a pivotal role in the export of organic anions, unconjugated bile acids and xenobiotics into the bile, and also contributes to protection against orally ingested drugs[26]. The phenotype associated with mutations in the gene encoding MRP2 is called Dubin–Johnson syndrome, a condition in which the lack of hepatobiliary transport of non-bile salt organic anions results in conjugated hyperbilirubinaemia[27]. MRP2 transports many of the same drugs as MRP1, with some notable differences (FIG. 3). Cells selected in cisplatin, arsenite or 9-nitro-camptothecin show increased MRP2 expression[28–31]. Although MRP2 has been detected in clinical specimens of cancers of renal, gastric, colorectal and hepatocellular origin, its expression has not been found to be predictive of response to chemotherapy.

Despite the similarity of their sequences, MRP3 transports fewer compounds than MRP1 or MRP2. Interestingly, MRP3 has a preference for glucuronides over GSH conjugates. Substrates of MRP3 include anticancer drugs and some bile acid species, as well as several glucuronate, sulphate and GSH conjugates[32]. MRP3 is mainly expressed in the kidney, liver and gut[33], which suggests a role for this protein in the enterohepatic circulation of bile salts. However, recent analysis of *mrp3*-deficient mice has not revealed any abnormalities in bile acid homeostasis, indicating that Mrp3 does not have a key role in bile salt physiology[34,35]. MRP3 expression has been observed in cancer tissues[36,37], and a correlation with doxorubicin resistance in lung cancer has been reported[38]. However, as MRP3 does not transport anthracyclines (FIG. 3), this correlation is not likely to be based on a causal relationship.

Intriguingly, mutations of the *MRP6* gene cause pseudoxanthoma elasticum, a systemic connective tissue disorder that affects elastin fibres of the skin, retina and blood vessels[39]. Studies indicate that MRP6-transfected cells become resistant to natural product agents, including etoposide, teniposide, doxorubicin and daunorubicin, whereas MRP7 is a resistance factor for taxanes[40,41]. As overexpression of MRP3, MRP6 or MRP7 has not been detected in resistant cell lines, their involvement in clinically relevant drug resistance or the physiological defence of tissues against xenobiotic compounds seems limited[42,43].

The ABCC subfamily contains four additional members that lack the N-terminal TMD. ABCC4 (MRP4), and ABCC5 (MRP5) confer resistance to nucleoside analogues such as 6-mercaptopurine and 6-thioguanine. Overexpression and amplification of the *MRP4* gene correlates with increased resistance to PMEA (9-(2-phosphonylmethoxyethyl)adenine) and efflux of azidothymidine monophosphate from cells and, therefore, with resistance to this drug[44]. The function of ABCC11 (MRP8) and ABCC12 (MRP9) is relatively unexplored. Cells overexpressing MRP8 are resistant to commonly used purine and pyrimidine nucleotide analogues[45] and to NSC 671136, a candidate anticancer drug tested against the NCI60 cancer cell panel[25]. In addition, MRP8 is thought to participate in physiological processes involving bile acids and conjugated steroids[46].

Phase II metabolic products
Cellular defence mechanisms against toxins are usually divided into several steps. ABC proteins hinder the cellular uptake of compounds (Phase 0). Should toxins enter the cells, they are subject to chemical modification (Phase I), and subsequent conjugation (Phase II). As a result of Phase I–II metabolism, toxins become more hydrophilic, and are expelled from the cells via mechanisms that involve ABC transporters (Phase III).

Enterohepatic circulation
Before entering systemic circulation, orally ingested drugs are directed to the liver via the portal vein. In the liver, drugs can be metabolized and sequestered to the gut. The enterohepatic circulation is an excretion–reabsorption cycle, in which drugs sequestered through the bile are reabsorbed in the gut.

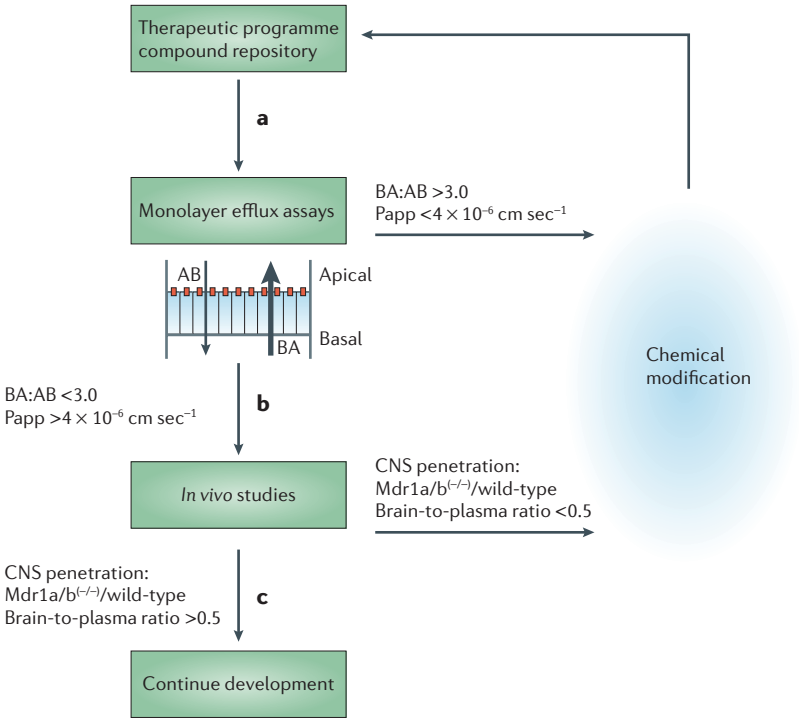

Figure 2 | **General scheme for evaluating P-glycoprotein susceptibility in early discovery and development of pharmaceutical drugs. a** | Passive permeability measured as the net apparent permeability (Papp) for compounds across polarized monolayers (for example, LLC-PK1 or Madin–Darby canine kidney II cells) in the absorptive (apical-to-basal; AB) and the secretory (basal-to-apical; BA) direction provides an indication of the capacity of a compound to access the systemic circulation when administered orally. A comparison of the BA:AB ratios obtained in parental cells and P-glycoprotein (Pgp)-overexpressing derivatives define the involvement of Pgp-mediated efflux. The BA:AB ratio observed in Pgp-overexpressing monolayers indicates the degree of Pgp-mediated efflux. Typically, BA:AB ratios of ≥3.0 suggest that the compound is a substrate of Pgp. However, the balance between Papp and the BA:AB ratio should be considered, as a compound with high permeability can overcome the active efflux. For compounds that have low permeability and/or high active efflux ratios, chemical modification could be required to ensure oral bioavailability. **b** | *In vivo* studies evaluating bioavailability can further define the systemic exposure of a compound, taking into consideration factors other than passive permeability (such as metabolism). Evaluating the brain-to-plasma ratio of compounds in *mdr1a/mdr1b (–/–)* and wild-type mice provides an indication of the capacity of the drug to penetrate the central nervous system (CNS). In case of limited exposure and/or low CNS penetration (depending on the therapeutic intent), chemical modification might be required. **c** | Compounds that have adequate Papp measures and limited Pgp susceptibility, as determined by *in vitro* and *in vivo* screens, would be considered for continued development.

Taken together, data from the literature indicate that several members of the ABCC (MRP) subfamily that have unrelated primary functions can be subverted for drug transport. However, it is still unclear whether experiments involving cells engineered to overexpress ABC transporters can be interpreted to suggest a general role for MRPs in clinical anticancer drug resistance.

ABCG subfamily. In contrast to most MRPs (with the possible exception of MRP1), ABCG2 (MXR/BCRP) clearly has the potential to contribute to the drug resistance of cancer cells. ABCG2, which is overexpressed in several cell lines selected for anticancer drug resistance, is a high-capacity transporter with wide substrate specificity. Transported substrates include cytotoxic drugs, toxins and carcinogens found in food products, as well as endogenous compounds[47,48]. Although several ABC transporters can transport methotrexate, ABCG2 has been shown to extrude glutamated folates, suggesting that it can provide resistance to both short- and long-term methotrexate exposure[49]. In addition, ABCG2 can transport some of the most recently developed anticancer drugs, such as 7-ethyl-10-hydroxycamptothecin (SN-38)[50] or tyrosine kinase inhibitors[51].

In all probability, the list shown in FIG. 3 will grow as new substrates or inhibitors are identified and additional ABC transporter proteins associated with decreased drug sensitivity of cancer cells are discovered. Screens carried out with the NCI60 cell panel indicate that there is a strong correlation between expression of several ABC transporters and decreased chemosensitivity, and also suggest that as many as 31 of the 48 ABC transporters could blunt the potency of the antitumour drugs screened in the study[25]. In addition, many other transporters, not related to the ABC family, potentially have a role in drug sensitivity and disposition. Experiments are underway to determine which of these can indeed confer drug resistance to tumours.

Significance of ABC transporters in cancer

Much has been learned about ABC transporters since MDR was first described[52]. Despite the wealth of information collected about the biochemistry and substrate specificity of ABC transporters, translation of this knowledge from the bench to the bedside has proved to be unexpectedly difficult. Of the transporters shown in FIG. 3, only inhibitors of Pgp, and to a lesser extent MRP1 and ABCG2, have been evaluated in clinical trials. *In vitro*, these three transporters efflux a broad range of chemotherapeutics used clinically for first- and second-line treatment of cancer. In that setting, inhibitors can often dramatically sensitize drug-resistant cell lines to known substrates. It is to be expected that this same effect would also occur *in vivo*. So, are ABC transporters important clinically, and does their inhibition translate into improved patient survival? Answers to the first part of this question come mainly from correlative studies evaluating the effect of Pgp expression on patient survival, whereas answers to the latter emanate from trials that combine chemotherapy with targeted inhibitors of Pgp-mediated drug transport.

Impact of ABC transporters on tumour response and patient survival. The role of ABC transporters in clinical anticancer resistance has been difficult to assess[53]. As is the case for most potentially useful cancer biomarkers, no universally accepted guidelines for analytical or clinical validation exist. Differences in tissue collection methodologies (for example, whole tissue versus laser-capture microdissection), molecular targets (for example, mRNA versus protein) and protocols have limited the ability to compare results across institutions. In addition, the absence of standardized criteria to score expression and effect has hampered adequate clinical validation.

Deciphering the impact of ABC transporter expression on patient survival is also challenging because of the

a

Drug class	Drug	ABC transporters overexpressed in cell lines selected for resistance						ABC transporters shown to confer drug resistance in transfection studies					
		ABCA2	ABCB1	ABCC1	ABCC2	ABCC4	ABCG2	ABCB11	ABCC3	ABCC5	ABCC6	ABCC10	ABCC11
Vinca alkaloids	Vinblastine		10,131	43,196	43							41	
	Vincristine		10	43,194	191							41	
Anthracyclines	Daunorubicin		10	43,194			137,211*				40		
	Doxorubicin		10,131	43,136	191		137,211*				40		
	Epirubicin		10	43	191		211*						
Epipodophyllotoxins	Etoposide		10	43	191		251		43,213,214		40		
	Teniposide		10				251		43,213,214		40		
Taxanes	Docetaxel		10		206							41	
	Paclitaxel		10		206			21				41	
Kinase inhibitors	Imatinib (Gleevec)		188	188			211						
	Flavopiridol						252						
Camptothecins	Irinotecan (CPT-11)		197,199,204	204	197–199	200,202	201						
	SN-38		197,199,204	204	198,199	200,202	201,210						
	Topotecan				198	202	203,210,211						
Thiopurines	6-Mercaptopurine					205				43			
	6-Thioguanine					205				43			
	5-FU									216			45
Other	Bisantrene		189				212						
	Cisplatin				191					216	40		
	Arsenite			43	207								
	Colchicine		10,131	195									
	Estramustine	181–183											
	Methotrexate		186	190,192,193	190,192	249	210,253*		43,193,213	215			
	Mitoxantrone	183	246	247	43		137,139,210						
	Saquinivir		185,187	248	248								
	PMEA					43,44,250				43,250			45
	Actinomycin D		10										
	AZT					208	217			208,209			

b

		ABCA2	ABCB1	ABCC1	ABCC2	ABCC4	ABCG2	ABCB11	ABCC3	ABCC5	ABCC6	ABCC10	ABCC11
First generation	Amiodarone		84										
	Cyclosporine		254	257	259		260	263				41	
	Quinidine		142,184	175									
	Quinine		69,236	175									
	Verapamil		14	257				263					
	Nifedipine		142										
	Dexniguldipine		227										
Second generation	PSC-833		254		259								
	VX-710 (Biricodar)		78	78			78*						
	GF120918 (Elacridar)		255				261						
Third generation	LY475776		223	223									
	LY335979 (Zosuquidar)		224										
	XR-9576 (Tariquidar)		226				222						
	V-104		221	221									
	R101933 (Laniquidar)		225										
Other	Disulfiram		220	258									
	FTC (Fumitremorgin C)						262						
	MK571			175	175	175			175	175		41	
	Tricyclic isoxazoles			228									
	Pluronic L61		256										

Figure 3 | **Substrates and inhibitors of ATP-binding cassette transporters. a** | Overlapping substrate specificities of the human ATP-binding cassette (ABC) transporters confering drug resistance to cancer cells. A single drug can be exported by several ABC transporters (rows), and each ABC transporter can confer characteristic resistance patterns to cells (columns). To determine which ABC transporters are involved in multidrug resistance (MDR), two different experimental procedures are common. Cells could be selected in increasing concentrations of a cytotoxic drug, which could result in the increased expression of a specific ABC transporter (see green boxes representing drug–gene pairs in which an ABC transporter was found to be overexpressed in cell lines selected for resistance to the respective drug). Resistant cells overexpressing a single ABC transporter often show characteristic cross-resistance to other, structurally unrelated, drugs (red boxes). Some ABC transporters were found to confer drug resistance only in transfection studies, in which cells are engineered to overexpress a given transporter. On transfection, cells become resistant to compounds that are substrates for transport (red boxes). White boxes denote unexplored or absent drug–gene relationships. **b** | The ability of ABC transporters to alter cell survival, drug transport and/or drug accumulation can be inhibited or altered by various modulators (yellow boxes). As in **a**, white boxes denote unexplored or absent drug–gene relationships. *The transport of these drugs by ABCG2 is dependent on an amino acid variation at position 482 (wild type is R; variants include R482G and R482T). Numbers in boxes refer to references. AZT, azidothymidine; 5-FU, fluorouracil; PMEA, 9-(2-phosphonylmethoxyethyl)adenine.

heterogeneity of tumours that have Pgp- and non-Pgp-mediated mechanisms of drug resistance. The resistance of tumours originating from tissues expressing high levels of Pgp (such as colon, kidney or the adrenocortex) often extends to drugs that are not subject to Pgp-mediated transport, suggesting that 'intrinsically resistant' cancer is also protected by non-Pgp-mediated mechanisms. Evidence linking Pgp expression with poor clinical outcome is therefore more conclusive for breast cancer, sarcoma and certain types of leukaemia, because Pgp-positive patients with these cancers can be compared with Pgp-negative patients of the same cancer type. As an example, a meta-analysis of 31 breast cancer trials showed a threefold reduction in response to chemotherapy among tumours expressing Pgp after treatment[54]. In another study, Pgp was found to be expressed in as many as 61% of pre-treatment soft tissue sarcomas (STS); even higher expression occurred following therapy with doxorubicin[55]. This is likely to be clinically important as doxorubicin is a known Pgp substrate and one of the main chemotherapeutic agents commonly used to treat STS. However, the validity of these findings remains controversial as Pgp positivity was variably defined throughout the trials, a limitation that is inherent to numerous studies assessing the impact of Pgp expression on patient survival.

In contrast to solid tumours, haematological malignancies are much easier to collect and purify. This relative sample homogeneity has allowed a more reliable determination of Pgp expression in leukaemic cells using techniques such as immunoflow cytometry and RT-PCR (reverse transcription-polymerase chain reaction). Functional assays, such as those using flow cytometry to measure efflux of fluorescent Pgp substrates (for example, Calcein-AM and rhodamine 123) from leukaemic cells, often complement expression analysis[56–58]. Using these techniques, more than a third of leukaemic samples are found to be positive for Pgp expression, and so the adverse impact of Pgp expression on patient survival or response rate has been most comprehensively evaluated for haematological malignancies, particularly acute myelogenous leukaemia (AML) and myelodysplastic syndrome (MDS). Pgp expression in patients with AML has consistently been associated with reduced chemotherapy response rates and poor survival, and it was found to be an independent prognostic variable for induction failure in adult AML[59,60].

Although compelling data exist indicating an important role for Pgp in determining efficacy of chemotherapy, the relevance of the other ABC transporters in clinical MDR is still unknown. MRP1 is not a significant factor in drug resistance in AML[61], and its prognostic implication in chronic lymphocytic and promyelocytic leukaemia, non-small-cell lung cancer (NSCLC) and breast cancer remains controversial[62–64]. Even less is known clinically about ABCG2 (REF. 65). Like adult stem cells, cancer stem cells express high levels of ABC transporters, including Pgp and ABCG2. According to the cancer stem cell model, this population of drug-resistant pluripotent cells defies treatment and serves as an unrestricted reservoir for drug-resistant tumour relapse[66]. Although ABCG2 is expressed in leukaemic $CD34^{+}38^{-}$ stem cells, its functional relevance seems limited[67].

Efforts to overcome MDR with Pgp inhibitors. The clinical importance of Pgp might also be determined through trials designed to abrogate Pgp function. Towards this end, less than 10 years after the discovery of Pgp-mediated MDR, the first Phase I and II clinical trials began to test the clinical potential of Pgp inhibitors. Initial trials used 'first-generation' Pgp inhibitors, including verapamil, quinine and cyclosporine (also known as cyclosporin A), which were already approved for other medical purposes. In general, these compounds were ineffective or toxic at the doses required to attenuate Pgp function. Despite these problems, a randomized Phase III clinical trial showed the benefit of addition of cyclosporine to treatment with cytarabine and daunorubicin in patients with poor-risk AML[68]. Similarly, quinine was shown to increase the complete remission rate as well as survival in Pgp-positive MDS cases treated with intensive chemotherapy[69], suggesting that successful Pgp modulation is feasible. However, several other trials failed to show improvement of the outcome and toxic side effects were common[70] (TABLE 1).

Promising early clinical trials encouraged further development. The second generation of inhibitors were devoid of side effects related to the primary toxicity of the compounds. For example, the *R*-enantiomer of verapamil and the cyclosporin D analogue PSC-833 (Valspodar) antagonized Pgp function without blocking calcium channels or immunosuppressive effects, respectively[71]. PSC-833 has been tested most frequently in clinical trials (TABLE 1), albeit with little success. Characteristic of the failures of second-generation inhibitors, PSC-833 induced pharmacokinetic interactions that limited drug clearance and metabolism of chemotherapy, thereby elevating plasma concentrations beyond acceptable toxicity. To preserve patient safety, empirical chemotherapy dose reductions were necessary; however, because pharmacokinetic interactions were generally unpredictable, some patients were probably under-dosed whereas others were over-dosed. Related to these problems, a Phase III trial using PSC-833 in previously untreated patients with AML who were >60 years old was closed early due to excessive mortality during induction in the experimental arm[72] (TABLE 1). A subsequent dose-escalation trial involving 410 patients with AML who were <60 years old revealed an overall survival advantage in an unplanned subset of patients of <45 years old[73]. That apparent benefit has not been duplicated, and it is unlikely to be, as development of PSC-833 has been discontinued. Similarly, development of another second-generation inhibitor showing initial promise (VX-710; biricodar) has been curtailed[74].

Third-generation inhibitors are designed specifically for high transporter affinity and low pharmacokinetic interaction. Inhibition of cytochrome P450 3A, which is responsible for many adverse pharmacokinetic effects with previous-generation inhibitors (BOX 3), has generally been avoided with the latest generation of inhibitors, including laniquidar (R101933), oc144-093 (ONT-093), zosuquidar (LY335979), elacridar (GF-120918)[75] and tariquidar (XR9576)[76]. Tariquidar has the added benefit of extended Pgp inhibition, as a single intravenous dose

Table 1 | **Characteristics and results of completed and Phase III clinical trials with ABC transporter inhibitors**

Year closed	Trial group	Number of participants	Cancer type	Modulator	Anticancer drugs	Dose reduced	Functional assay	Outcome	Refs
1992		223	Breast	Quinidine	Epirubicin	No	No	No benefit	229
1993		68	NSCLC	Verapamil	Vindesine, Ifosfamide	No	No	Improved OS	230
1993		226	SCLC	Verapamil	CAVE	No	No	No benefit	231
1995		200	Myeloma	Verapamil	VAD	No	No	No benefit	232
1995		130	SCLC	Megestrol acetate	CAV/EP	No	No	No benefit	233
1995	MRC	235	Relapsed and refractory AML	Cyclosporine	ADE	No	No	No benefit	234
1995	HOVON, MRC (C302)	428	AML	PSC-833	Daunorubicin, cytarabine, etoposide	No	Yes	No benefit	235
1996	GFM	131	High-risk MDS	Quinine	Mitoxantrone, cytarabine	No	No	Improved OS in Pgp-positive patients	69, 236
1996	Novartis (C301)	256	AML	PSC-833	Mitoxantrone, etoposide, cytarabine	No	No	No benefit	237
1996		315	Poor-risk acute leukaemia	Quinine	Mitoxantrone, cytarabine	No	Yes	No benefit	238
1998	SWOG	226	Poor-risk AML, RAEB-t	Cyclosporine	Daunorubicin, cytarabine	No	Serum	Improved OS in cyclosporine group	68
1999	GEO-LAMS	425	*De novo* AML	Quinine	Idarubicine, cytarabine, mitoxantrone	No	Yes	Significant improvement in the CR rate in Pgp-positive patients. No OS advantage	239
1999	CALGB (9720)	120 (age >60 years)	Untreated AML	PSC-833	Daunorubicin, etoposide, cytarabine	Yes	No	Terminated early owing to secondary toxicity	72
2000		238	Advanced and recurrent breast cancer	MS-209	Cyclophosphamide, doxorubicin, fluorouracil	–	–	No benefit	240
2000	CALGB (9621)	410 (age <60 years)	Untreated AML	PSC-833	Daunorubicin, etoposide, cytarabine	Yes	No	No OS advantage for those >45 years; survival benefit for those <45 years	73
2000		99	Breast	Verapamil	Vindesine, 5-FU	No	No	Improved OS and RR	242
2001	EORTC, HOVON	81	Myeloma	Cyclosporine	VAD	No	No	No benefit	237
2002		762	Ovarian	PSC-833	Carboplatin, paclitaxel	Yes	–	No benefit	241
2003	ECOG (E2995)	144	Refractory AML, high-risk MDS	PSC-833	Mitoxantrone, etoposide, cytarabine	Yes	–	No benefit	243
2003		304	NSCLC	PSC-833	Carboplatin, paclitaxel	Yes	–	Terminated early owing to secondary toxicity	‡
2003	CALGB (19808)	302	AML	PSC-833	IL-2	No	–	Results pending	§
2005	ECOG	450	AML, MDS	LY335979	Daunorubicin, cytarabine	No	Yes	Results pending	§

–, Unknown. ‡Novartis; §Cancer.gov. 5-FU, fluorouracil; ADE, cytarabine, daunorubicin and etoposide; AML, acute myelogenous leukaemia; CAVE, cyclophosphamide, doxorubicin, vincristine and etoposide; CAV/EP, alternate treatment with CAV regimen and a combination of cisplatin and etoposide; CR, complete response; IL, interleukin; MDS, myelodysplastic syndrome; NSCLC, non-small-cell lung cancer; OS, overall survival; Pgp, P-glycoprotein; RAEB-t, refractory anaemia with excess of blasts in transformation; RR, response rate; SCLC, small-cell lung cancer; VAD, vincristine, adriamycin and dexamethasone.

inhibited efflux of rhodamine from $CD56^+$ cells (biomarker lymphoid cells that express Pgp) for at least 48 hours[77]. Several later-generation inhibitors act on multiple ABC transporters (FIG. 3). Biricodar (VX-710) and GF-120918, for example, bind Pgp as well as MRP1 and ABCG2, respectively[78]. Although affinity for multiple drug transporters might extend the functionality of these inhibitors to Pgp-negative tumours showing MDR, the scope of possible side effects also increases. In 2002, Phase III clinical trials began using tariquidar as an adjunctive treatment in combination with first-line chemotherapy for patients with NSCLC. Despite the promising characteristics mentioned above, the studies were stopped early because of toxicities associated with the cytotoxic drugs (a full explanation for trial closure is not available)[79]. This study also illustrates a defect in experimental design, as there is no strong evidence to suggest that NSCLC expresses Pgp to a significant extent (BOX 4). Following the review of the aborted trials, the National Cancer Institute (NCI) has commenced further exploratory Phase I/II and Phase III studies with tariquidar. Zosuquidar has recently been evaluated in patients with AML. Preliminary analysis indicates that zosuquidar can be safely given without chemotherapy dose reductions (L. D. Cripe, personal communication); trial endpoints have not yet been analysed.

Although Pgp is clearly established as a prognostic marker in adult AML, after more than three decades of research, the clinical benefit of modulating Pgp-mediated MDR is still in question. This is, in part, due to limitations of candidate inhibitors, and the inadequate design of the trials[80] (BOXES 3,4). Although most trials using first- and second-generation inhibitors give reason to doubt the benefit of Pgp modulation, the verdict is still out. Clearly, the inhibitors used today are much improved from those used in the past, with greater substrate specificity, lower toxicity and improved pharmacokinetic profiles. Results from Phase III trials using third-generation inhibitors will be pivotal in determining whether inhibition of Pgp, or other ABC transporters, can result in improved patient survival.

Clinical trials have distilled the concept of an ideal transporter antagonist. The perfect reversing agent is efficient, lacks unrelated pharmacological effects, shows no pharmacokinetic interactions with other drugs, tackles specific mechanisms of resistance with high potency and is readily administered to patients. This might be too much to ask from a cancer drug that targets a network of transporters with a pivotal role in ADMET. In more realistic terms, the ideal inhibitor should restore treatment efficiency to that observed in MDR-negative cases. Nevertheless, modulators are unlikely to improve the therapeutic index of anticancer drugs unless agents that lack significant pharmacokinetic interactions are found[81]. The search for such 'fourth generation' inhibitors is ongoing, and there is no shortage of compounds showing *in vitro* sensitization of MDR cells. Similar to their predecessors, some of the emerging candidates are 'off the shelf' compounds (old drugs with new tricks), such as disulfiram, used to treat alcoholism[82], or herbal constituents[83] shown to inhibit Pgp function *in vitro* in concentrations that are compatible with clinical applicability. Recent developments in pharmacology, such as the introduction of HTS technology and 'screen-friendly' synthetic chemical libraries, combined with improved understanding of substrate–protein interactions[84] should enable rational planning and *de novo* synthesis of novel Pgp modulators[85]. In addition to traditional pharmacological modulation, more creative approaches have emerged in the literature. These strategies to engage, evade or even exploit efflux-based resistance mechanisms are discussed in the next section (FIG. 4).

Alternative approaches to targeting MDR

Peptides and antibodies that inhibit Pgp. Pgp-mediated drug resistance can be reversed by hydrophobic peptides that are high-affinity Pgp substrates. Such peptides, showing high specificity to Pgp, could represent a new class of compounds for consideration as potential chemosensitizers[86]. Small peptides corresponding to the transmembrane segments of Pgp act through a different mechanism. Peptide analogues of TMDs are believed to interfere with the proper assembly or function of the target protein, as was shown in experiments aimed at the *in vitro*[87] or *in vivo*[88] inhibition of G-protein-coupled receptors. Small peptides designed to correspond to the transmembrane segments of Pgp act as specific and potent inhibitors, suggesting that TMDs of ABC transporters can also serve as templates for inhibitor design[89]. Studies suggest that immunization could be an alternative supplement to chemotherapy. A mouse monoclonal antibody directed against extracellular epitopes of Pgp was shown to inhibit the *in vitro* efflux of drug substrates[90]. Similarly, immunization of mice with external sequences of the murine gene *mdr1* elicited antibodies capable of reverting the MDR phenotype *in vitro* and *in vivo*, without eliciting an autoimmune response[91].

Targeted downregulation of MDR genes. Selective downregulation of resistance genes in cancer cells is an emerging approach in therapeutics. Although in cell lines MDR is often a result of the amplification of the *MDR1* gene, the overexpression of the protein has transcriptional components as well. Regulation of Pgp expression is amazingly complex, and could include different mechanisms in normal tissues compared with cancer cells[92]. If mechanisms governing expression of Pgp in malignant cells were mediated through tumour-specific pathways, cancer-specific approaches to circumvent Pgp overexpression could be developed with minimal effect on constitutive expression of normal cells[93]. Using peptide combinatorial libraries, Bartsevich *et al.*[94] designed transcriptional repressors that selectively bind to the MDR1 promoter. Expression of the repressor peptides in highly drug-resistant cancer cells resulted in a selective reduction of Pgp levels and a marked increase in chemosensitivity[94,95]. Similarly, antagonists of the nuclear steroid and xenobiotic receptor (SXR), which coordinately regulate drug metabolism and efflux, can be used in conjunction with anticancer drugs to prevent the induction of Pgp[96]. Using technologies that enable the targeted regulation of genes — antisense oligonucleotides, hammerhead ribozymes and short-interfering RNA

Box 3 | **Possible reasons for failure in Phase III trials targeting P-glycoprotein**

Potential reasons for the failure of compounds that target P-glycoprotein (Pgp) in Phase III trials include[142]:

Alternative mechanisms of resistance

Unfavourable pharmacological properties of the inhibitors:

- Low affinity (ineffective inhibition)
- Poor specificity (unrelated pharmacological activity)
- Low bioavailability at tumour site

Toxicity of the inhibitors:

- Primary toxicity of the first- and second-generation reversing agents (for example, hypotension, ataxia and immunosuppression)
- Secondary toxicity due to inhibition of Pgp in physiological sanctuaries such as bone marrow stem cells

Pharmacokinetic interactions[143]*:*

- Pgp modulators can decrease the systemic clearance of anticancer drugs, thereby increasing exposure to normal and malignant cells and so potentially increasing the severity and/or incidence of adverse effects associated with the anticancer therapy[144].
- There is a considerable overlap in the substrate specificities and regulation of cytochrome P450 3A (CYP3A) and Pgp. CYP3A, the major Phase I drug-metabolizing enzyme, and Pgp have complementary roles in intestinal drug metabolism, where, through repeated extrusion and reabsorption, Pgp ensures elongated exposure of the drugs to the metabolizing enzyme[145]. Inhibition of Pgp can interfere with CYP3A-mediated intestinal or liver metabolism, resulting in reduced drug clearance.
- Interaction with other ATP-binding cassette (ABC) transporters, such as ABCB4 and ABCB11, which results in compromised biliary flow[146].

Empirical dose-modification of chemotherapy:

- To accommodate expected elevations in systemic drug exposure, some patients might have been over-dosed or under-treated.

(siRNA) — has produced mixed results. Sufficient down-regulation of Pgp has proved difficult to attain and the safe delivery of constructs to cancer cells *in vivo* remains a challenge[97,98]. However, transcriptional repression is a promising new strategy that is not only highly specific but also enables the prevention of Pgp expression during the progression of disease.

Novel anticancer agents designed to evade efflux[15]. Several novel anticancer drugs are exported by ABC transporters, including irinotecan (and its metabolite SN-38), depsipeptide, imatinib (Gleevec; Novartis) and flavopiridol (FIG. 3). Moreover, the NCI60 screen suggests that a significant portion of the compounds in the drug development pipeline are substrates of ABC transporters[25,53]. Epothilones are novel microtubule-targeting agents with a paclitaxel-like mechanism of action that are not recognized by Pgp, providing proof of the concept that new classes of anticancer agents that do not interact with the multidrug transporters can be developed to improve response to therapy. As most anticancer agents subject to efflux are currently irreplaceable in chemotherapy regimens, an attractive solution would be to chemically modify their susceptibility to being transported while retaining antineoplastic activity. Although such modifications frequently decrease the bioavailability or efficacy of drugs, some new agents have been developed using this approach[99]. The intracellular concentration of drugs can also be elevated by increasing the rate of influx. This 'apparent circumvention' of Pgp-mediated efflux can be achieved by increasing the lipophilicity of compounds (positive charge and degree of lipophilicity dictate, or at least influence, whether compounds are recognized by MDR1) or by stealth formulations. For example, highly lipophilic anthracycline analogues[100], such as annamycin and idarubicin, were shown to elicit a high remission rate in Pgp-positive AML cases with primary resistance to chemotherapy[101]. The efficacy of these drugs is currently being evaluated in the MRC AML15 trial[59]. Encapsulation of doxorubicin in polyethylene glycol-coated liposomes (PLD) might be safer and occasionally more effective than conventional doxorubicin[102]. PLD was found to cross the BBB, and seemed to overcome the MDR of tumours in preclinical models. The combination of this formulation with PSC-833 suppressed tumour growth to an even greater degree in mouse xenograft models, providing proof-of-principle for Phase I studies[103,104]. A clever approach combines drugs encapsulated in polymeric micelles with ultrasound treatment of tumours. As a consequence of the encapsulation, the systemic concentration and cellular uptake of the drug decreases, reducing unwanted side effects. To trigger drug release, the tumour is irradiated with ultrasound[105].

Theoretically, the simplest way to counter efflux mechanisms is to increase drug exposure of cancer cells through prolonged or higher-dose chemotherapy. Indeed, it could well be that the benefit of classical inhibitors was derived solely from the augmented dose intensity of the concomitantly administered chemotherapeutics, as opposed to the pharmacodynamic modulation of target cells[106]. Unfortunately, the therapeutic window of anticancer agents is very narrow, as even a slight increase in chemotherapy dosages results in potentially lethal side effects.

Exploiting drug resistance by protection of normal cells. A major dose-limiting factor of standard chemotherapy is bone-marrow toxicity. When transferred to haematopoietic cells, Pgp was shown to protect the bone marrow, suggesting the feasibility of chemotherapeutic regimens at formerly unacceptable doses[107]. This approach can also be used in stem-cell-based gene therapy, as the co-expression of a drug-resistance protein with a therapeutic gene product in genetically modified stem cells allows both the *in vitro* enrichment of the corrected cells and *in vivo* drug selection during clinical gene therapy. Another strategy to selectively protect normal cells is based on drug combinations that include a cytotoxic and a cytoprotective agent[108]. In the presence of the protective agent, normal cells remain unharmed, whereas MDR cells, which pump out the protective agent, succumb to the cytotoxic therapy ('unshielding of MDR cells'). For example, the non-Pgp-substrate apoptosis-inducing agent flavopiridol was shown to selectively kill Pgp-expressing cells when used in combination with the caspase-inhibitor Z-DEVD-fmk, which is pumped out from MDR cells[109].

Exploiting drug resistance by targeting MDR cells with peptides and antibodies. Ideally, therapy is directed against specific target cells. MDR cancer cells are eminent targets for destruction, and the high surface expression of Pgp could be exploited in strategies that use antibodies to

Box 4 | **Scheme of Phase III clinical trial design targeting ABC transporters**

The following steps could be used to improve the design of Phase III clinical trials for agents that target ATP-binding cassette (ABC) transporters[147,148]:

Step 1: Assessing the impact of ABC transporters on drug resistance
Define and standardize methods and the scoring system to be used to determine whether a tumour expresses the ABC transporter of interest. Such standardized scoring systems have been successfully implemented in the case of other targeted therapies (that is, determination of HER2/*neu* and oestrogen receptor status for breast cancer therapy with trastuzumab and hormonal agents, respectively[53]). This requires rigorous analytical validation of all reagents, measurement technologies and tissue collection/storage procedures for all participating research sites[149,150].

Step 2: Defining target patient groups
Enrol patients most likely to respond. Ideally, randomized trials should be undertaken, using large, meticulously profiled patient populations. As the beneficial effect of transporter inhibition will probably be confined to patients 'positive' for the transporter target, adequate transporter expression and/or function should be a criterion for trial enrolment. The targeted transporter(s) should be expressed at levels previously determined to have an adverse effect on prognosis. ABC transporter expression or function of haematological malignancies can be readily determined *ex vivo* using either immunoflow cytometry or fluorescent drug substrate efflux assays, respectively. Similarly, solid tumours can be evaluated for expression of ABC transporters using either mRNA or protein-based technologies; functional imaging using ^{99m}Tc-sestamibi would be complementary.

Step 3: Choice of appropriate treatment protocols
Because inhibitors have no inherent anticancer activity, they must be coadministered with cytotoxic agents. Improvement of therapy outcome is expected only if the chemotherapeutic regimens involve transported substrates. Chemotherapy drug combinations should be used at concentrations previously proven safe and effective in Phase I/II trials, taking into account potential pharmacokinetic interactions with either the parental drug compound or its metabolites.

Step 4: Monitoring drug levels and side effects
Drug pharmacokinetics and early signs of hepatic, neurological or bone marrow toxicity should be monitored closely.

Step 5: Monitoring efficacy by surrogate assays
To ensure abrogation of the multidrug-resistant phenotype, surrogate assays should be carried out to assess the effect of the inhibitor in each patient. This can be done either *ex vivo*, by using flow cytometry to measure P-glycoprotein (Pgp) function in CD56$^+$ cells taken from patients treated with inhibitors, or '*in vivo*' using ^{99m}Tc-sestamibi[151] or other imaging modalities to directly image accumulation of Pgp substrates within tumours.

bridge effector molecules and cells. Anti-Pgp antibodies have been successfully used to destroy Pgp-expressing cells in antibody-mediated cytolysis experiments, and have also been used as immunotoxins[110,111]. More recently, Morizono *et al.*[112] have used a mouse melanoma model engineered to express the human *ABCB1* gene to show that metastatic cells can be successfully targeted with a vector linked to an anti-Pgp monoclonal antibody. Immune response to the anti-Pgp immunoglobulins and the toxic side effects expected in normal tissues expressing Pgp are concerns that have to be addressed before the widespread clinical use of these strategies. Future enhancements of the technology, such as the replacement of the monoclonal antibodies with peptide fragments, will be important for successful clinical applications.

Exploiting the paradoxical sensitivity of MDR cells. Gene expression studies have shown that MDR cells can be profoundly different from their sensitive counterparts[31]. Perhaps as a result of these differences, MDR cells that are cross-resistant to structurally and functionally unrelated drugs can simultaneously show paradoxical hypersensitivity to certain compounds. MDR cells were found to be collaterally sensitive to membrane-active agents such as the calcium-channel blocker verapamil; inhibitors such as PSC-833 or LY294002 (REFS 113–115); and various stress-inducing compounds, including 2-deoxy-D-glucose[116,117], tunicamycin and 5-fluorouracil[118,119].

In an effort to catalogue compounds against which MDR cells might show collateral sensitivity, we characterized the expression profile of the 48 ABC transporters in the NCI60 cancer cell panel[25]. The NCI60 cell panel was set up by the Developmental Therapeutics Program of the NCI to screen the toxicity of chemical compound repositories[120]. We explored the relationship between ABC transporter expression levels and sensitivity to drugs or drug candidates, asking which of the transporters confer resistance or sensitivity to various classes of agents. In particular, we searched for statistical correlations between the cell lines' sensitivity to cancer drugs and the expression of ABC transporters. Using this pharmacogenomic approach, we identified strongly correlated 'drug–gene' pairs, in which the expression of an ABC transporter, most notably MDR1/Pgp, correlated with increased sensitivity to a drug. This correlation suggested that the toxicity of several compounds can be potentiated, rather than antagonized, by the MDR1 multidrug transporter. Follow-up studies have verified that cells become hypersensitive to 'MDR1-inverse' compounds, such as NSC 73306, in proportion to their Pgp function. The physiological function of Pgp includes transmembrane transport of a broad spectrum of endogenous substrates, some of which have a role in regulation of cell growth. Recent observations support the possibility that Pgp can promote cell survival by efflux-independent pathways, including the inhibition of caspase-dependent apoptosis[121] or the reduction of ceramide levels through either the reduction of inner leaflet sphingomyelin pools or the modulation of the glucosylceramide synthase pathway[122,123]. In view of these findings, it can be speculated that downstream changes in the apoptosis-inducing pathways in MDR cells might be responsible for the preferential susceptibility to MDR1-inverse compounds[119]. Cells expressing other ABC transporters could become similarly sensitive. For example, increased MRP1 expression could be accompanied by the intracellular depletion of important molecules, such as GSH, resulting in an increased susceptibility to oxidative stress[124].

Conclusions

An ultimate goal in cancer therapy is to devise individually tailored treatment that targets growth-promoting pathways and circumvents drug resistance. In considering how to go about cataloguing important mechanisms of drug resistance in cancer, it makes sense to begin by focusing on the family of ABC transporters, as they are widely expressed in cancer cells and their capacity to confer drug resistance has been established, at least *in vitro*. Pgp represents one of the best-studied mechanisms of resistance to hydrophobic anticancer drugs. It remains to be seen whether other ABC transporters will emerge as culprits for treatment failure.

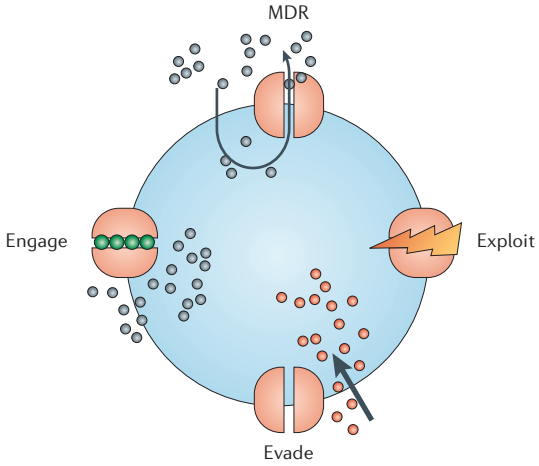

Figure 4 | **Targeting multidrug-resistant cancer.** P-glycoprotein (Pgp) actively extrudes many types of drugs from cancer cells, keeping their intracellular levels below a cell-killing threshold. Strategies that circumvent Pgp-mediated multidrug resistance (MDR) include the co-administration of pump-inhibitors and cytotoxic agents ('engage') and the use of cytotoxic agents that bypass Pgp-mediated efflux ('evade'). A third approach takes advantage of the collateral sensitivity of MDR cells ('exploit').

Despite the clear rationale for the use of inhibitors of ABC transporters, especially of Pgp, the development of these products and demonstration of their efficacy has been slow. With a lack of marketable products, pharmaceutical companies have begun to lose interest. Only a few compounds are currently in clinical trials, as the development of most of the inhibitors (including valspodar (PSC-833), dexniguldipine, dextroverapamil and biricodar (VX-710)) has been discontinued. The bottleneck seems to be the unwelcome inhibition of ABC transporters at pharmacologically important locations. However, as more and more information about pharmacokinetic effects accumulate, new-generation inhibitors become more specific and potent (as shown through careful Pgp measurements and surrogate biological markers of Pgp inhibition). Ultimately, we anticipate that the efficacy of ABC transporter modulation will be established in a subset of human cancers. A clear-cut demonstration of the effectiveness of targeting Pgp will result in renewed interest and the development of further ABC transporter inhibitors will follow suit.

In the meantime, several new therapeutic modalities can be explored using existing inhibitors. Most of the clinical trials have been carried out in patients with prior therapies, in whom acquired resistance is likely to have developed through multiple mechanisms. It could well be that ABC transporters have a role in the initial phases of tumour evolution, to provide a window of opportunity for the cancer cells to develop alternative mechanisms of resistance. To test this hypothesis, clinical trials could be carried out to assess the possibility of preventing, rather than fighting, MDR cancer[106]. ABC transporter modulators could also be used to influence the oral bioavailability or increased CNS penetration of drugs[125]. Studies should also address the significant heterogeneity associated with individual responses to pharmacological treatment, in particular the role of inherited traits in limiting drug disposition. It is reasonable to assume that genetic variations in ABC transporters have profound effects on pharmacokinetics. The clinical relevance of Pgp polymorphisms has been intensively studied, and a synonymous mutation (C3435T) has been shown by some laboratories to be associated with altered protein expression and consequent changes in drug disposition[126]. C3435T is part of a haplotype that might contribute to this altered drug-transport phenotype, but most studies are not sufficiently statistically powered to give convincing results. Despite the controversy, some consider Pgp to be a prominent example of the effectiveness of pharmacogenomics in associating polymorphisms with clinically relevant variables.

The enormous effort of cancer biologists and pharmacologists to understand MDR in cancer has resulted in the identification of a limited number of distinct, clinically proven mechanisms. Overexpression of ABC transporters, particularly Pgp, has consistently been implicated as a cause for MDR both *in vitro* and *in vivo*. Recent strategies to engage, evade or exploit this transporter to improve cancer treatment reflect both the creativity and hopefulness of cancer researchers that at least this cause of MDR can be vanquished.

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Acknowledgements
We thank G. Leiman for his excellent editorial assistance.

Competing interests statement
The authors declare no competing financial interests.

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